MS-MS approaches for the analysis of environmental pollutants

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INTRODUCTION:

Concern about the environment and the start of environmental analysis coincided with the rise of gas chromatography-mass spectrometry (GC-MS). The United States Environmental Protection Agency (U.S. EPA) was founded in 1970, and as the need for techniques to analyze environmental samples for pollutants was becoming evident, the application of mass spectrometry to the separation provided by gas chromatography and facilitated through the use of computerized systems was found to be almost a perfect match for doing the job. Therefore, for the next ten to fifteen years, GC-MS was the workhorse for environmental analyses. Especially for target analysis, whereby a specific, known compound was looked for in environmental samples, GC-MS had no peer. Relying on the efficient separation of volatile or semi-volatile compounds in the GC and the reproducible ionization effected in the MS, methods could be standardized, and regulatory strictures could be written for toxic compounds in the environment. GC-MS methods are still widely used today, but as regulators became more and more concerned with polar and non-volatile compounds, it was seen that these methods were not able to detect many compounds of interest that were being considered for regulation. Indeed, many of the early pollutants were put on regulatory lists because they could be detected by GC-MS. That is no longer a restriction.

To analyze samples for polar and non-volatile compounds, liquid chromatography - especially high performance liquid chromatography (HPLC) - was seen as a technique to separate these intractable compounds. However, interfacing the HPLC to the MS was not an easy engineering problem, and this union lagged behind the GC-MS. After several attempts to build an interface capable of removing the liquid effluent from the HPLC and make it conducive to the vacuum region of the MS, researchers in the mid or late 1980s made exciting breakthroughs in liquid chromatography-mass spectrometry (LC-MS). The two major LC-MS techniques that were responsible for this advancement are thermospray and its successor electrospray. These techniques revolutionized mass spectrometry by opening up the field of polar and non-volatile pollutants (and biological substances) that could be analyzed.

A key point in this discussion is that both thermospray and electrospray are ionization techniques, in addition to being interfaces between the LC and the MS. This is important, since GC-MS relies on the electron impact ionization technique to ionize the molecules that come from the GC into the mass spectrometer. This ionization, usually undertaken at 70 eV, is very reproducible and affords fingerprint identification of the specific compound. Many libraries of electron impact ionization mass spectra have been compiled, and if one operates the instrument under standard conditions, one can easily identify the compounds by their mass spectra. The confirming piece of information is running the GC-MS of the reference standard of the pollutant and having the retention time (on the GC axis) and the fragmentation pattern (on the MS axis) match up. However, thermospray and electrospray ionize the molecules under softer conditions. Thus, instead of producing many ions per compounds and producing the fingerprint indicative of a certain compound, these techniques produce few ions per compound and in many cases just

one ion per compound is produced - usually associated with the molecular weight of that compound. This makes identification of unknowns impossible and of target compounds suspect. Given the fact that LC retention times are not so precise as those generated under GC conditions, there was need for a method to deconvolute the information stored in the ion(s) produced by thermospray or electrospray.

MS-MS

Instrumentation

High Resolution Mass Spectrometry

A solution to the problem of getting useful information for the ions produced in the LC-MS interfaces was found in the various manifestations of what has come to be known as mass spectrometry-mass spectrometry (MS-MS) or tandem mass spectrometry. This concept was originally applied to high resolution mass spectrometers to study collision energy transfer and energy release in the dissociation of activated ions (Shukla, Qian, Anderson, and Futrell, 1990). In fact, the process of collisionally activated dissociation (CAD), sometimes known as collisionally induced dissociation (CID) came to play an important part in these tandem mass spectrometers (McLafferty, et al.,1973). These high resolution instruments, usually consisting of two sectors (an electric and magnetic), were decoupled for their use in the tandem mass spectrometer, as one sector was used to focus an ion of interest and the other to focus the ions that were created in the CAD process. A reverse geometry high resolution instrument of BE (B

magnetic field; E electric field) configuration was used for the first MS-MS experiments (Busch and Cooks, 1983). Other kinds of MS-MS scans could be performed in high resolution instruments by linking interactions between the accelerating field, the magnetic field, and the electric field of the instrument. MS-MS was first applied not to LC, but to GC, fast atom bombardment, field ionization, and other techniques for getting the compounds into the mass spectrometer and ionizing them.

Triple Quadrupole Mass Spectrometry

The use of tandem mass spectrometry for environmental analysis benefited from the development of the triple quadrupole mass spectrometer; the high resolution instruments of various configurations were mainly focused on fundamental studies. While GC-MS for environmental use relied mainly on the single quadrupole mass filter instrument, the GC-MS-MS and later LC-MS-MS used three quadrupole mass filters to perform the analysis (Yost and Enke, 1978, 1979). Figure 1 is a schematic of the triple quadrupole MS. The first quadrupole was configured to filter the ions of interest by allowing only one ion through the quadrupole. The second quadrupole exists mainly as a collision cell being operated in the radiofrequency (RF)-only mode. The quadrupole region is enclosed in a structure that allows pressure to be maintained, so that the ion of interest from the first quadrupole can interact with the (usually) inert gas in the collision cell. An offset voltage is used in conjunction with the collision cell, so that the collision can be at an energy to fragment the ion of interest. Being operated in the RF-only mode allows all the ion products of the collisions to be transmitted through the cell. It also allows focusing of the product ions to minimize losses to the walls of the cell. Sometimes

higher-order mass filters are used (hexapoles and octopoles) to add better focusing power to the collision cell, thus increasing the transmission. The third quadrupole is usually operated in the normal mode of scanning the voltages and producing a mass spectrum of the ions generated in the collision cell. What has been described above has been called the product ion scan (formerly called the daughter ion scan), which has been the most popular scan of the triple quadrupole instrument. Other major scan modes used in the operation of the triple quadrupole mass spectrometer are the neutral loss scan and the precursor ion scan. In the neutral loss scan quadrupoles one and three were scanned in tandem offset by a certain mass range corresponding to a neutral loss that was expected in the collision cell (e.g., 28 mass units from a predicted loss of CO). This scan indentifies all those compounds that lose a common neutral. The precursor ion scan (formerly called the parent ion scan) was undertaken by scanning the first quadrupole and having the third quadrupole set to allow just one ion through. This scan captured those compounds that generated the same ion (e.g., m/z 149 for phthalates).

Ion Trap Mass Spectrometry

The instruments described above are classified as tandem mass spectrometers (or MS-MS) in space. These techniques rely on a sequential number of processes, which the ion(s) encounters as they transverse the instrument. Alternatively, there are other instruments that are classified as tandem mass spectrometers in time. The most widely used instrument in this class is the quadrupole ion trap. All the processes occur in the same physical space in the ion trap, but are separated according to a time sequence. As the name indicates, ions are trapped by the voltages and the geometry of the ring electrode of the ion trap (see Figure 2). Helium is present in the ion

trap to cool the ions so that they reside mainly in the middle of the trap. The ion trap is very adaptable to the MS-MS process so the same hardware is used for MS-MS experiments as is used for full scans. There is just the matter of having the correct electronics and scan functions to operate in MS-MS mode. In fact, helium, which is already present, can also be used as a collision gas to effect fragmentation in the CAD process.

Briefly, the precursor ion of interest is retained after ionization and trapping, but the other trapped ions are ejected by either applying a broadband waveform or ramping the RF voltage. Since there are continuous collisions between the helium and the ions, which do not result in broken chemical bonds, the translational kinetic energy of the precursor ion must be increased to the point where bonds are broken through an increase in the vibrational energy. This energy must not be too large or the precursor ion will be ejected. There are two ways of increasing this translational kinetic energy – non-resonant excitation and resonant excitation. A waveform applied to the ion trap produces the excitation. The non-resonant excitation is easy to apply, but it is not selective on which ions to excite. The second form of excitation (resonant excitation) is difficult to perform, since it must match the oscillation frequency of the precursor ion. However, it does excite the specific ion of interest (Varian, 2007). This process may be repeated with one of the fragment ions produced by the CAD process isolated as above and this ion is excited by the same process. Therefore, one can do this routine a number of times to produce an MS^n (MS-MS-MS…) spectrum (de Hoffmann, 1996; Creaser and Stygall, 1998), whereby fragments ions from one MS-MS experiment are the precursors for another MS-MS experiment, and so on. The ions of interest are then ejected from the trap and are detected at the electron multiplier.

The product ion scan is the only scan that can be obtained with the ion trap, so no neutral loss or precursor ion scans, but as mentioned above the product ion scan can be repeated to produce the MS^n spectrum. In addition to providing multiple steps of fragmentation, the overall CAD process on the trap operates at very high efficiencies (80-90%) (Creaser and Stygall,1998, Johnson, Yost, Kelley and Bradford, 1990).

Time of Flight Mass Spectrometry

For tandem mass spectrometry, the time of flight (ToF) mass spectrometer is not a stand alone instrument, but one used with other analyzers that separate the precursor ion and effect fragmentation. What the ToF instrument offers is high resolution, good mass accuracy, high sensitivity, and fast scanning (Hager and Le Blanc, 2004) as the second stage of mass spectrometry. Usually, when the ToF instrument is used with a quadrupole mass filter on the front end, the ToF instruments are configured in an orthogonal geometry configuration, which allows a decoupling of the ion velocity on injection into the ToF (Hager and Le Blanc, 2004, Guilhaus, Selby and Mlynski, 2000). This decoupling allows a low initial velocity, which results in very good resolution and a linear mass calibration scale. The end results are very good mass assignment accuracies, which are usually less than 5 ppm (Lee, Monte, Sanderson and Haskins, 1999).

The ToF instrument has been used mostly with quadrupole mass filters (e.g., q-ToF or QqToF), but have also been used with three-dimensional quadrupole ion traps on the front end (Michael, Chien, and Lubman, 1992). Thus, MS^n spectra can be acquired before the fragments are injected

into a ToF instrument. This combination is especially useful for structure elucidation, as the MS^n feature can give a sequential fragmentation pattern that the analyst can use to determine the overall structure. Having a ToF on the back end with its high resolution capabilities can give exact mass information for these fragment ions, which gives an extra measure of clarity to this structure identification exercise.

Linear Ion Traps

The use of tandem mass spectrometry, when interfaced with LC introduction, has some drawbacks, especially when ion traps are implemented. The three-dimensional ion trap and an LC interface, such as electrospray ionization (ESI) source, do not couple efficiently because the ESI source is continually producing ions and the ion trap works in the pulsed mode and is also bothered by an abundance of ions. There is a potential loss of sensitivity, plus the duty cycle of the instrument is lower than it could be in a continuous environment (Cox, Cleven and Cooks, 1995; Wilcox, Hendrickson and Marshall, 2002). Therefore, the idea of a linear quadrupole ion trap was proposed to solve some of these problems (Cha, Blades and Douglas, 2000). First of all, the linear quadrupole system has a higher transport efficiency than static lenses at fixed voltages, plus it can operate at higher pressures (good for the interface of atmospheric pressure and the low vacuum of the mass spectrometer). In addition, by applying blocking potentials to the entrance and exit, ions can be stored in the linear quadrupole with RF only applied to its rods (Cha, Blades and Douglas, 2000). Now the linear ion trap has also become a trapping device of greater capacity than the three-dimension ion trap without the problem of space charging from the abundance of ions. Furthermore, ions of selected mass-to-charge ratios (m/z) can be excited

at their resonant frequencies for ejection or fragmentation. Therefore, isolation of the analyte ion of interest from any matrix ions is effected. The result is that the signal-to-noise ratio improves, in addition to mass resolution, and the duty cycle increases to nearly 100% (Senko, et al., 1997). The linear ion trap can then be used as the first device in a tandem mass spectrometer, especially if the spectrometer is being used with ESI. For example (see Figure 3), the linear quadrupole ion trap is being used with a three-dimensional ion trap in a tandem mass spectrometer. Conversely, the linear ion trap has been used as the final mass spectrometer in the tandem MS with quadrupoles for the precursor ion selection and fragmentation in the collision cell region (Hager and Le Blanc, 2003). In this arrangement, there have been reports of increased sensitivity by over 500 times that of the standard triple quadrupole (Hager and Le Blanc, 2003).

Orbitrap

A relatively new entry in the field of instruments that trap ions to generate mass spectra is the orbitrap. Insufficient mass accuracy of the three-dimensional ion trap and the prohibitive cost of other trapping instruments (e.g., FT ICR) prompted work on the orbitrap, which uses dynamic ion trapping in electrostatic fields (Makarov, 2000). As is the case with three-dimensional ion traps, the orbitrap benefits from the use of linear ion traps between itself and the ESI source, i.e., it requires accumulation of ions in an external device and a gated transfer of the ions into the orbitrap (Hardman and Makarov, 2003); see Figure 4. When the orbitrap is combined with the linear ion trap, this mass spectrometer is capable of achieving mass resolution of 150,000 full width at half maximum (FWHM), mass accuracies of about 1-3 ppm, and a mass range of 2000 daltons (Hardman and Makarov, 2003).

ENVIRONMENTAL APPLICATIONS OF MS-MS

One of the first applications of MS-MS to environmental samples was a clever scheme to screen samples quickly using the various scans of the triple quadrupole (Hunt, Shabanowitz, Harvey and Coates, 1985). Instead of using the standard method of extraction, concentration, and separation by chromatography, Hunt, Shabanowitz, Harvey and Coates (1985) placed solid matrices and freeze-dried aqueous samples on glass wool plugs in glass tubing and then heated the samples via the solid probe. They set up eight experiments consisting of one precursor ion scan and seven neutral scans and looked for all of the priority pollutants. Instead of the many hours for a complete analysis, this scheme was completed in 25 minutes. For fairly clean samples, this method showed that it was good for screening. However, the need for a derivatization step and the threat of very dirty samples relegated this method to academic use.

The use of tandem mass spectrometry with electron impact ionization was demonstrated in two studies using ion trap technology (Nourse, Cox, Morand and Cooks, 1992; Pyle, et al.,1997). Each study involved polyaromatic hydrocarbons (PAH), a suite of compounds, which has been monitored carefully by the U.S. EPA. Nourse, Cox, Morand and Cooks (1992) used the MS^n capabilities of the ion trap to follow the gamut of CAD reactions, which can take place with pyrene and anthracene. In this way, the authors wanted to delineate the fragment pathways taking place in both compounds and thus demonstrate structure elucidation on these compounds and eventually for other “unknown” compounds. Since these compounds were introduced on a solids probe, plenty of material was present to perform MS-MS experiments multiple times. In fact, they were able to demonstrate MS^n, where n=10. Pyle, et al. (1997) used GC introduction

of a group of PAHs into the ion trap. They were able to demonstrate the resonant and nonresonant modes of the ion trap. In the resonant mode, the applied frequency matches the oscillation frequency of the trapped precursor ion and fragmentation will result only from that specific ion. In nonresonant excitation, the applied frequency is not matched to the oscillation frequency of the trapped ion and the kind of fragmentation that results is not discriminative and multiple collisions of fragmented ions can occur. The resulting spectra show more fragmentation than a resonant spectrum (Pyle, et al.,1997). A nice example of both modes and a comparison to the CAD on a triple quadrupole mass spectrometer using pyrene as an example is show on Figure 5.

The advent of effective and reliable liquid chromatographic interfaces made the use of tandem mass spectrometry imperative. Since the successful LC interfaces of thermospray and presently electrospray are soft ionization techniques, CAD techniques were needed to deconvolute the structural information present in the one or two ions present per compound in the spectra generated from the interfaces. These CAD techniques were an integral part of the tandem mass spectrometers developed just previously to the invention of these successful LC interfaces.

The first applications of tandem mass spectrometry to environmental analysis with thermospray ionization used a triple quadrupole mass spectrometer to identify dyes (Betowski and Ballard, 1984; Ballard and Betowski, 1986). Dyes are compounds of interest to the U.S. EPA, both because of their large-scale production and the potentially hazardous chemicals used in their production and the byproducts that can be formed. Dyes were not routinely analyzed for by GC-MS methods because of their ionic structure and nonvolatility. Thermospray was very useful in

getting several classes of dyes into the mass spectrometer in an ionic form; electrospray would later prove successful in getting more classes of dyes amenable for mass spectrometric analysis (Bruins, Covey and Henion, 1987; Bruins, Weidolf, Henion, and Budde, 1987). A dye of unknown structure and several potential precursors were found in a thermospray spectrum in this early work (Betowski and Ballard, 1984). With the triple quadrupole mass spectrometer, Betowski and Ballard (1984) were able to obtain collision spectra of the MH^+ ions, and therefore, piece together the structure of the parent dye and also of the precursors that were in the dye formulation. Thus, this can be considered one of the first forensic applications of tandem MS to the environment.

Emerging Contaminants

The terms emerging contaminants or emerging pollutants have been used recently by environmental chemists. The term does not necessarily mean that these compounds classified as emerging were recently added to commerce and were being introduced to the general public. (Daughton, 2001). This may be the case, but the main reason these compounds receive this classification is because the analytical tools have been created so these compounds may now be identified. At the head of the list of new techniques are the LC interfaces that enable mass spectrometry to be used on samples containing these once intransigent compounds. With these new interfaces, especially electrospray, comes the need for CAD and MS-MS techniques to interpret the results. In addition to tandem mass spectrometers, there has been an effort to create a low-cost CAD instrument, in which there is but a single analyzer (quadrupole, ToF, etc.). In this option, in-source CAD is performed, which effectively raises the potentials in the ion source

region, which are at an elevated pressure, to produce indiscriminant collisions of all the ions present. Where there are few matrix effects, this is a viable alternative to tandem instruments. However, since a specific ion is not targeted, the results are sometimes inconclusive as to what product ions come from what precursor ions.

There are many classes of emerging compounds. One of the first groups of compounds to be classified emerging was endocrine disrupting chemicals. These compounds, as the name indicates, interfere with the functioning of the endocrine system and affect the balance or production of hormones in the body (Colborn, vom Saal, and Soto,1993). Prime examples of endocrine disrupting compounds that are classified as emerging are the various estrogens. They can come from natural or artificial origins. For example, 17 β-Estradiol, a natural estrogen, is present in females in higher concentrations than in males. Synthetic estrogens, such as ethynyl estradiol, are used extensively for contraceptive and therapeutic purposes (Sparrow, 1987; Diaz-Cruz, Lopez de Aldea, Lopez.and Barceló, 2003). Diethylstilbestrol (DES) has also been used extensively in estrogenic hormone therapy in the prevention of miscarriage in humans and as a growth promoter in livestock (Martindale, 1982). Wastewater treatment plants have not been optimized for removal of these compounds; thus estrogens enter the aquatic environment and can reach concentrations normally in the nanogram and subnanogram per liter level (Rodriguez-Mozaz, Lopez de Alda and Barceló, 2004). These compounds, furthermore, have been implicated in the feminization of wild fishes living downstream from wastewater effluent (Sole, et al.,2003; Sumpter,1995).

The analysis of samples for estrogens has been performed using enzyme-linked immunosorbent assay (ELISA) (Huang and Sedlak, 2001; Mitani, Fujioka and Kataoka, 2005) and GC-MS or GC-MS-MS) (Kelly, 2000; Nakamura, Sian and Daishima, 2001; Xiao, McCalley and McEvoy, 2001). However, ELISA results tend to overestimate the concentrations (because of cross reactivity), and GC-MS methods require derivatization steps, which complicate the analysis. Therefore, LC-MS and LC-MS-MS are used more frequently for the analyses of estrogens. Rodriguez-Mozaz, Lopez de Alda and Barceló (2004), and Mitani, Fujioka and Kataoka (2005) have coupled LC methods with on-line devices for sample preparation and pre-concentration techniques for the analysis of estrogens. By the use of solid phase extraction (Rodriguez-Mozaz, Lopez de Alda and Barceló, 2004) or solid phase micro-extraction (Mitani, Fujioka and Kataoka, 2005) techniques, these groups were able to simplify the analysis of estrogens with a high throughput operation. The setup for the Kataoka group (2005) is seen in Figure 6. Both groups acquired data in the negative ion mode and by selected reaction monitoring (SRM) (Rodriguez-Mozaz, Lopez de Alda and Barceló, 2004) or by multiple reaction monitoring (MRM) (Mitani, Fujioka and Kataoka, 2005). SRM is the technique analogous to selected ion monitoring in GC-MS in which one ion is focused in the first quadrupole, allowed to react with the collision gas in the second quadrupole, and one of the product ions is selected for detection in the third quadrupole. Multiple reaction monitoring allows more than one product ion to be detected. Limits of quantitation (LOQ) using these systems are as low as 0.02 to 1.02 ng/L (Rodriguez-Mozaz, Lopez de Alda and Barceló, 2004).

A word of caution must be added when using LC-MS-MS. When analyzing complex environmental samples, both false negative and false positives can result, if the work is not carefully done. False negative results can occur due to ionization suppression effects, while false

positives can be due to insufficient selectivity (Reemtsma, 2001). The criteria that has been used to limit the occurrence of both false negative and positive results are as follows: (1) the retention time must be within one to two percent of the standard, and (2) the relative abundances of at least two ions selected for SRM must be within 20% of the ion ratios for the standards (Baronti, et al., 2000; Lagana, Bacaloni, Fago and Marino, 2000; Lopez de Alda, Diaz-Cruz , Petrovic and Barceló, 2003).

Pharmaceuticals

Since many of the compounds listed as emerging contaminants are pharmaceuticals or more correctly, active pharmaceutical ingredients (APIs), they merit some discussion. In addition, pharmaceuticals are meant to be beneficial to humans or animals; therefore, their listing as contaminants is complicated. The main threat could be ecological damage, which is focused on the adverse effects of myriad APIs on marine life. Since APIs are constantly being input into the marine environment, mainly through discharge from wastewater treatment, plants, fish and other marine organisms are always bathed in this soup (Daughton and Ruhoy, 2009).

In analysis for APIs, it is important to know the concentrations in water, such as the outflow of a wastewater treatment plant, but it is also instructive to know the concentrations in sludges or sediments to which APIs can sorb from the water phase. Two examples of these analyses were by Ternes, et al. (2005) on sludges and Gebhardt and Schröder (2007) on wastewater. Ternes, et al. (2005) were interested in determining several APIs, including the antiepileptic carbamazepine, cytostatic agents, the psychiatric drug diazepam and also iodinated contrast

media in activated and digested sludge. Extraction was performed by either ultrasonic solvent extraction or pressurized liquid extraction. Cleanup was undertaken with RP-C18ec material and silica gel. Electrospray (for the neutral APIs and the iodinated contrast media in the positive ion mode) or atmospheric pressure chemical ionization (APCI) (for the acidic APIs in the negative ion mode) was interfaced with a triple quadupole mass spectrometer for the determinative analysis. The data were acquired in the multiple reaction monitoring mode in which a precursor ion was focused in quadrupole one and the product ions from CAD reactions in quadrupole two were focused in quadrupole three. Either one or two product ions were monitored per compound. Limits of quantitation for these compounds were reported as 20-50 ng/L (Ternes, et al., 2005). Gebhardt and Schröder (2007) were interested in following the fate of carbamazepine, diazepam, diclofenac, and clofibric acid through various wastewater treatment processes. They used an LTQ Orbitrap MS for the analysis, using ESI introduction with both positive ion and negative ion modes. The system was operated in both the normal scan mode, with a resolution of 300 mmu FWHM, and in the CAD mode, with high resolution (60,000 at m/z 400) and high mass accuracy full scan MS-MS mass spectra. Figure 7 shows a good example of how the LTQ Orbitrap, operating in the CAD mode at high resolution, high mass accuracy was able to pick out the ion of interest in a complex wastewater sample. Operating in the negative ion mode, this instrument was able to identify clofibric acid and diclofenac, respectively, by their SRM masses (Gebhardt and Schröder, 2007). These compounds were continually present in the outfall of the local wastewater treatment plant at low, but easily detectable levels (ng per liter).

Pesticides and Herbicides

Chloroacetanilides, triazines, phenylureas, and some of their metabolites were determined by GC-MS-MS, LC-MS, and LC-MS-MS (Dagnac, et al., 2005). Ion trap systems were used in conjunction with the GC system and one of the LC systems (in the tandem mode). A single-quadrupole MS system was used for the other LC system. Furthermore, APCI was used with the LC-single quadrupole MS system, and both ESI and APCI were used with the LC-ion trap system in the MS-MS mode. Therefore, it is instructive to compare the LOQs for these herbicides using these various mass spectrometers. Table 1 shows a direct comparison for LOQs for these compounds on each of these instruments (Dagnac, et al., 2005). The single quadrupole LC-MS system, as expected, shows poorer results than the GC-MS-/MS and LC-MS-MS systems. Linuron is much better detected with APCI than with ESI. APCI generally detects the neutral compounds better than ESI, which is excellent for acidic or basic compounds.

Even with the benefit of LC and tandem mass spectrometry, some samples are so contaminated with matrix materials (humic acids, tissue materials, etc.) that good extraction and cleanup methods are needed prior to LC-MS-MS. Interference in the ionization process by compounds in the matrix material is a problem in quantification of target compounds. To try to circumvent this problem Gervais, Brosillon, Laplanche and Helen (2008) reported the use of SPE with ultra-high-pressure liquid chromatography (UPLC) before analysis by tandem mass spectrometry for 31 pesticides in river water. The use of ultra-high pressure methods affords LC the best peak separation and resolution. In doing so, competition between target compounds and matrix material is minimized. A triple quadrupole MS was used as the tandem mass spectrometer. In

394 the method development process using the triple quadrupole MS, care must be taken to optimize
395 the cone voltages and collision energies to maximize the signals of the precursor ion and the
396 product ions, respectively. An example of this optimization process is shown in Figure 8 for the
397 pesticide diflufenican. Using this method, the LOQs for 31 pesticides, analyzed in either the
398 positive or negative mode, were between 10 and 51 ng/L (as indicated in Table 4 from Gervais,
399 Brosillon, Laplanche and Helen, 2008).
400
401 The use of SPE sorbents can still retain extraneous materials during the extraction process.
402 These materials could enhance or suppress electrospray ionization signals. García-Galán, Díaz-
403 Cruz and Barceló (2010) incorporated molecularly imprinted polymers (MIP) into the SPE
404 process for better affinity and selectivity for triazines and their metabolites analyzed under
405 tandem MS conditions. The authors found that the MIP used in the extraction process proved
406 suitable for cleanup and concentration of complex soils. The quadrupole linear ion trap MS used
407 for the analysis was found suitable for this work and resulted in high sensitivities, as shown by
408 LODs in the low ng/L and ng/g region (García-Galán, Díaz-Cruz and Barceló, 2010).
409
410 ***DNA Adducts and Oxidative Stress Markers of Environmental Pollutants***
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412 Risk assessments of environmental pollutants can be simplified by measuring the DNA-adducts
413 of these compounds in human tissues. By measuring these adducts, one has physical evidence of
414 damage to the DNA that is not available by only measuring the parent pollutant compound. By
415 the use of HPLC/ESI/MS-MS and isotopic dilution, Means, Olsen and Schoffers (2003) were
416 able to measure DNA adducts of aromatic amines at 100 femtomolar levels, which corresponds
417 to one adduct in approximately 6×10^7 normal nucleotides. The use of isotopic dilution

minimized any quantification errors, whereby the same precursor to product ion reaction was monitored in both the actual adduct and the isotope counterpart.

Feng, Wang, Yuan and Wang (2009) took this isotopic dilution approach further in their analysis of stereoisomers of anti-benzo[a]pyrene diol epoxide (BPDE) adducts by incorporating UPLC MRM using a triple quadrupole MS. Anti-BPDE is the ultimate carcinogen from the PAH benzo(a)pyrene, and it can form stereoisomeric DNA-adducts with deoxyguanosine (dG) or deoxyadenosine(dA) (Willems, 2002). The use of MRM on the triple quadrupole MS makes the analysis specific enough that interfering BPDE tetrols are no longer a problem in the separation of four stereoisomeric anti-BPDE–N2dG adducts. The use of UPLC with MRM MS-MS was able to shorten the run time from 60-100 minutes to 2-4 minutes. Besides saving time, the peaks were sharper and more concentrated. This fact plus the use of stable labeled isotopes for quantification made for a very fast and sensitive detection for the four stereoisomer adducts (Feng, Wang, Yuan and Wang, 2009). This method will be useful to help evaluate the metabolic activation and detoxification of benzo[a]pyrene, and DNA adduct formation and repair at low doses.

Because of its higher sensitivity and specificity, and the ability to determine multiple analytes, LC-MS-MS has become a favored choice for looking at oxidative stress markers over other techniques. Oxidative stress in the body has been implicated in disease states, but the actual oxidative chemicals (free radicals, etc.) are hard to measure in situ. Therefore, oxidative stress compounds in bodily fluids, such as blood, serum, and urine have been studied as markers of disease (Winnik and Kitchin, 2008). One of the most common class of markers of oxidative

441 stress comprises the isoprostanes, which are linked to lipid peroxidation. F2-isoprostanes are
442 prostaglandin F2-like species formed by free radical oxidation of arachidonic acid and have been
443 recognized as a measure of oxidative stress (Winnik and Kitchin, 2008). Immunochemistry
444 methods for these compounds may suffer from cross reactivity with other prostaglandin
445 metabolites and may, therefore, be unreliable. Other isoprostanes, like F4-neuroprostanes, are
446 also indicators of oxidative stress, neurodegenerative disease in this example. Yan, Byrd and
447 Ogden (2007) described an LC-MS-MS method for several isoprostanes using solid phase
448 extraction, the negative ion mode, and a deuterium-labeled isoprostane. A semi-automatic LC-
449 MS-MS method was developed by Haschke, et al. (2007) that included an extraction cartridge
450 on-line with the LC for F2-isoprostane quantification in plasma and urine. A useful LC-MS-MS
451 method with SRM was developed for biologically significant thiols, which are involved in
452 pathological processes. These thiols were monitored in mouse liver tissue (Bouligand, et al.,
453 2006). Another LC-MS-MS SRM method was devised for detecting elevated levels of
454 homocysteine, an oxidative stress marker associated with stroke and other diseases (Tomaiuolo,
455 et al., 2006). Aromatic amino acids, when oxidized, can yield oxidative stress markers. The
456 measurement of these markers may provide information about the nature and origin of damage
457 caused by oxidative stress (Orhan, Coolen and Meerman, 2005). 3-Nitrotyrosine is an end
458 product that arises from the oxidation of tyrosine by various oxidizing species *in vivo*. Ryberg
459 and Caidahl (2007) indicated that 3-nitrotyrosine is a biomarker for over 40 different diseases
460 and determined that the high selectivity and sensitivity of LC-MS-MS and the fact that
461 derivatization was not needed made it an ideal method for quantifying low levels of 3-
462 nitrotyrosine in human plasma.
463

Surfactants and Dyes

As discussed earlier, dyes were some of the first analytes to be investigated by LC-MS-MS methods. Many of them are ionic compounds, which necessitates the use of LC methods. Surfactants are another class of compounds that is characterized by high polarity and are amenable to analysis by LC. Surfactants are ubiquitous in wastewater, even after treatment. They started to be recognized as a problem when LC/MS-MS methods began to be applied to wastewater analysis. While the treatment of wastewater eliminated most of the nonpolar compounds, surfactants remain. Schröder (1993) was one of the first to analyze for surfactants by LC-MS-MS, by applying triple quadrupole MS-MS methods to thermospray ionization. The surfactants can be classed in two categories, ionic and non-ionic. The non-ionic surfactants are characterized by ions separated by m/z 44 units or m/z 58 units, which refer to glycol ether chains (Schröder, 1993). The ionic surfactants are often of the sulfonic acid category, which were detected in the negative thermospray ionization mode.

There has been much interest in the monitoring the fate of textile dyes in wastewater streams (Betowski, Pyle, Ballard and Shaul, 1987). This interest comes from both an aesthetic point of view (to avoid colored water from the water tap) as well as environmental and health concerns (Clayson, 1962; Hueper, 1969). Therefore, a continuous on-line sampling method would be a real benefit for those monitoring the efficiency of the wastewater treatment process for commercial dyes. Plum and Rehorek (2005) incorporated HPLC with diode array detection (DAD) and ESI-MS-MS into a continuous monitoring system to be used as a process diagnostic tool to reveal information about the biologically mediated azo dye reduction. Since ion-pairing

reagents were used for chromatography with anionic dyes, a cationic suppression technique was used before the eluent went into the ESI-MS. Non-volatile ionic species tend to block the ESI interface, plus suppress the signal of the ions of interest. The use of a linear ion trap quadrupole LC-MS-MS system allowed the monitoring of the starting materials, as well as unknown intermediates released in the anaerobic and aerobic treatment process (Plum and Rehorek, 2005).

Ozone

Monitoring ozone is approached from two sides: (1) measuring the actual ozone levels, and (2) measuring the products of reaction with ozone. These two approaches present a different problem from the measurement of ozone in the stratosphere that protects from harmful UV radiation. Since ozone is a powerful oxidizing agent, it is potentially harmful to human health through effects on biomolecules. For example, the buildup of oxidized proteins has been related to various diseases, such as cataracts and rheumatoid arthritis (Pirie, 1971; Kotiaho, Eberlin, Vainiotalo and Kostiainen, 2000). In a study on the aqueous ozonation of 22 amino acids and some small peptides using ESI-MS-MS, Kotiaho, Eberlin, Vainiotalo and Kostiainen (2000) were able to identify products of this process. Their success was due to the ability of ESI-MS-MS to identify product ions at low levels. Of the 22 amino acids, only four formed oxidation products that were observed by ESI-MS, histidine (His), tryptophan (Trp), tyrosine (Tyr), and methionine (Met). They further found that the order of reactivity with ozone was Met>Trp>Tyr>His. In the small peptides that were studied, the same amino acids were involved in the oxidation process, but the order of reactivity changed slightly: Met>His>Trp>Tyr. The products of ozone oxidation in these peptides were so unique, such that ozone oxidation plus

analysis by ESI-MS-MS has been proposed in determining the structure of unknown peptides (Kotiaho, Eberlin, Vainiotalo and Kostiainen, 2000).

Another study brought together the use of ozone in wastewater treatment with a pharmaceutical of interest, tetracycline (Dalmázio, Almeida and Augusti, 2007). Because of its wide use in both human and animal treatment against infectious diseases, tetracycline can be found in the influent/effluent of wastewater treatment plants (Karthikeyan and Meyer, 2006). The use of ozone was thought to be an advanced treatment for wastewater and, therefore, able to mineralize tetracycline and similar compounds. This proved not the case, however, as there was no evidence of formation of by-products by extensive ring breakdown. The use of ESI-MS^n (with ion trap technology) was able to detect and characterize two important highly polar intermediates (Dalmázio, Almeida and Augusti, 2007).

SUMMARY

The use of MS-MS or tandem mass spectrometric techniques has revolutionized chemical/biological analysis. The creation of electrospray by Professor John Fenn was worthy of a Nobel Prize (Fenn, et al.,1989), but the utility of this technique would not be so widespread, had it not been for MS-MS. One of the areas touched greatly by these inventions has been the environmental field. The success of environmental analyses benefited by the introduction of GC/MS, and the maturity of the technique of GC/MS developed out of its use to characterize environmental samples. It was then appropriate that these analyses would benefit also from the use of LC-MS-MS and coincidentally also promote the development of this technique. We

have learned that there have been various manifestations of tandem mass spectrometry, each unique in its own way, and each lending to the usefulness of MS-MS. These manifestations include reversed geometry high resolution instruments, triple quadrupole MS, ion traps MS of various sorts, various hybrid instruments, such of the quadrupole-TOF MS, and most recently orbitraps. Each of these instruments has enabled the use of structural information to either identify the compound or allow the quantification of that compound by targeting specific ion pairs in the CAD process. The environmental uses of these techniques have run the gamut from toxic pollutants of pesticides and herbicides to emerging contaminants, such as pharmaceutical and personal care products, or analyzing biological materials that contain DNA-adducts of toxic compounds or oxidative stress markers as a result of pollutant contamination. These techniques have enabled the most sensitive analyses for pollutants, while maintaining specificity not available from other instrumentation. In fact, in many cases the analytical instrumentation has exceeded the detection limits where toxicity stops being a danger. However, the problem arises about the toxicity of many compounds at sub-toxic levels. Do these pollutants exert synergy that make the mix a toxic soup? These are questions to be answered as the analytical instrumentation gets more and more refined.

NOTICE:

The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to Agency's administrative review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

TABLE

1. Limits of quantification (µg/kg) for herbicide determination by GC-MS-MS and LC-MS/(MS). (Taken from Dagnac, et al. (2005), with permission from Elsevier.)

Limits of quantification (µg/kg) for herbicide determination by GC–MS/MS and LC–MS(/MS)

Herbicide	GC–MS/MS	LC–APCI/MS	LC–ESI/MS/MS	LC–APCI/MS/MS
Atrazine	1.9	5.4	0.15	0.3
DIA	1.6	22	4.5	11
DEA	2.5	13	0.7	1.2
Metolachlor	0.3	6.3	0.3	0.3
Acetochlor	0.9	22	0.7	1.5
Alachlor	2.2		1.5	1.5
DIPU		22	6	1.5
MIPU		11	0.7	0.7
Isoproturon		4.8	0.7	3
Chlortoluron		10	7.5	3
Linuron			7.5	0.7
Diuron			4.5	4.5

2. FIGURE CAPTIONS

1. Operational schematic of the TQMS. Sample gas goes from left to right. The circled letters represent molecules and molecular fragments. (Taken from Brand and Gregg (1989), figure 1, with permission, LLNL.)
2. Quadrupole ion trap schematic. (Creaser and Stygall (1998), figure 1, with permission from Elsevier).
3. Schematic diagram of the linear quadrupole interface. The IQ orifice (interquad orifice) serves as an entrance control and L_1 serves as an exit control for Q_1. L_2 focuses ions from Q_1 into the 3D trap. (Cha, Blades and Douglas (2000), figure 1, with permission from the American Chemical Society.)
4. Schematics of the orbitrap interfaced with electrospray. (Hardman and Markov (2003), figure 1, with permission from the American Chemical Society.)
5. Ion-trap nonresonant (a) and resonant (b) spectra of pyrene and TSQ700 spectrum of pyrene (c). (Pyle, et al. (1997), figure 3, with permission from Elsevier.)
6. Schematic diagram of the on-line in-tube SPME/LC/MS-MS system. (Mitani, Fujioka and Kataoka (2005), figure 2, with permission from Elsevier.)
7. Elution of pharmaceuticals. (a) LC–ESI(-)-MS total-ion current tracing for the C18-SPEconcentrated feed extract of Aachen-Soers treatment plant. (b) Extracted mass trace of clofibric acid. (c) Extracted mass trace of diclofenac. (d) LC–ESI(-)-MS-MS product-ion mass trace of clofibric acid (tR: 8.83 min; clofibric acid concentration: 0.02 ng ml-1). (e) LC–ESI(-)-MS-MS product-ion mass trace of diclofenac (tR: 10.29 min; diclofenac concentration: 1.5 ng ml-1). (Gebhardt and Schröder (2007), figure 3, with permission from Elsevier.)

8. Signal intensity of the different product ions of the pesticide diflufenican versus collision energy , data acquired in MRM mode (ESI+). (Gervais, Brosillon, Laplanche and Helen (2008), figure 2, with permission from Elsevier.)

Figure 1.

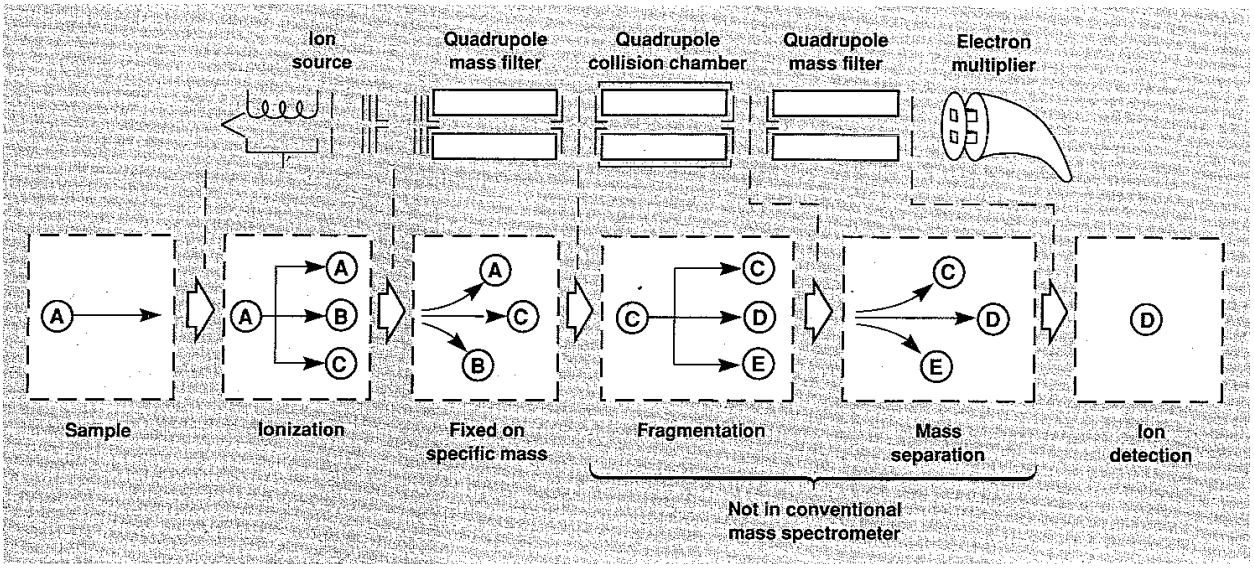

Figure 2.

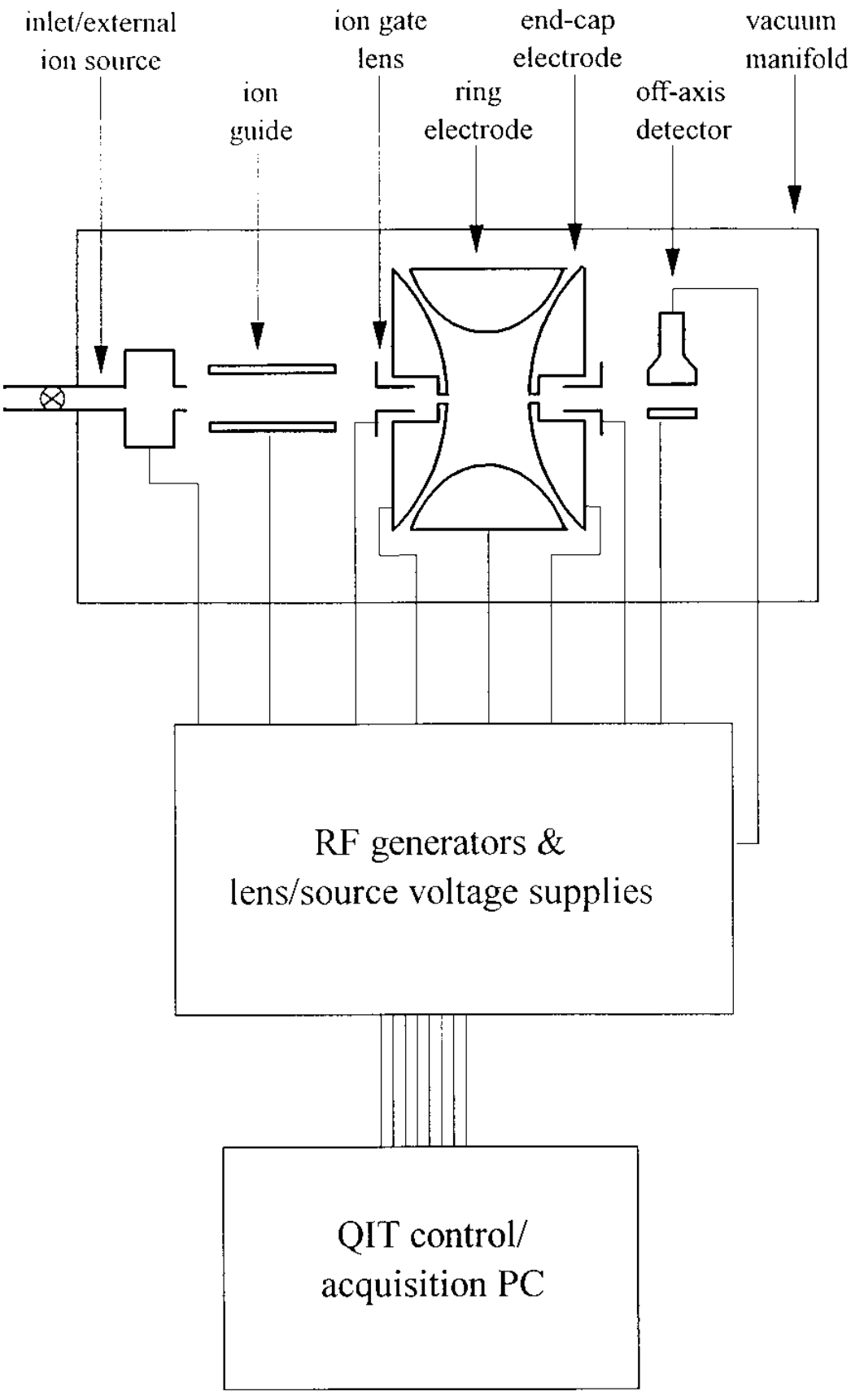
inlet/external
ion source
ion
guide
ion gate
lens
ring
electrode
end-cap
electrode
off-axis
detector
vacuum
manifold
RF generators &
lens/source voltage supplies
QIT control/
acquisition PC

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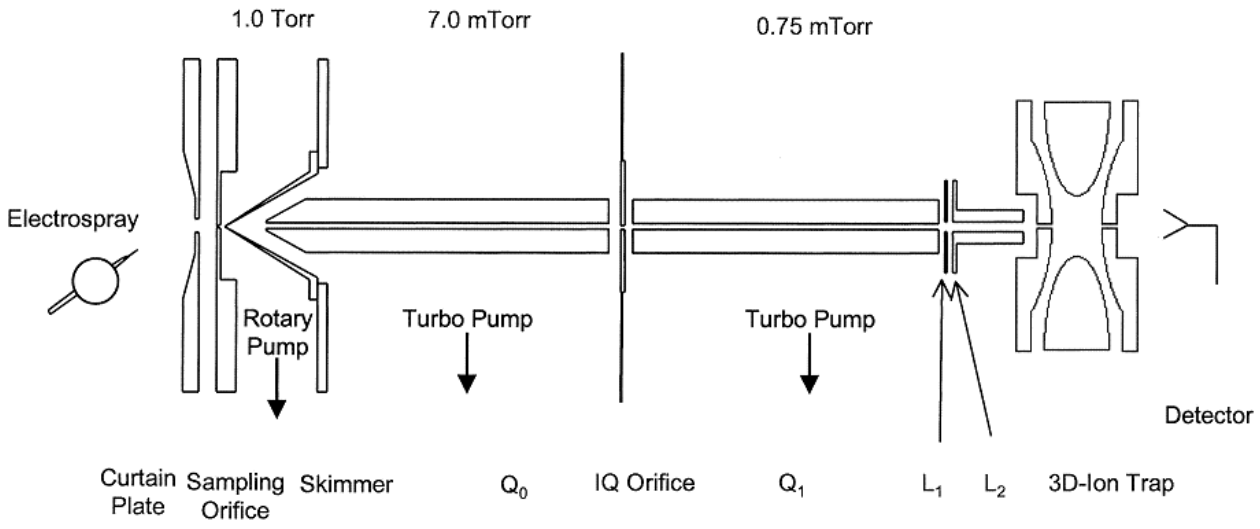
Figure 3.


1.0 Torr
7.0 mTorr
0.75 mTorr
Electrospray
Rotary Pump
Turbo Pump
Turbo Pump
Detector
Curtain Plate
Sampling Orifice
Skimmer
Q_0
IQ Orifice
Q_1
L_1
L_2
3D-Ion Trap

645 Figure 4.
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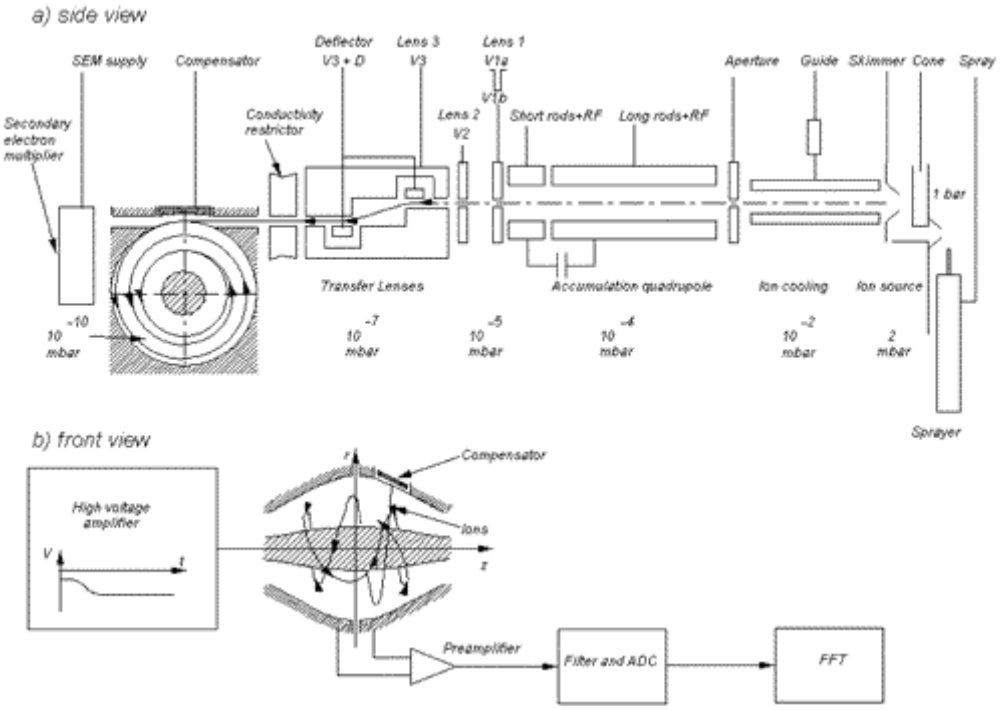

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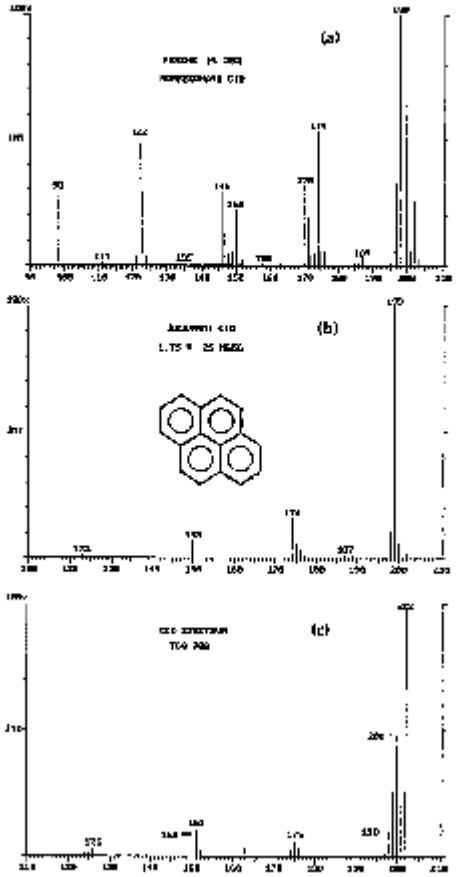
Figure 5.



667 Figure 6.
668
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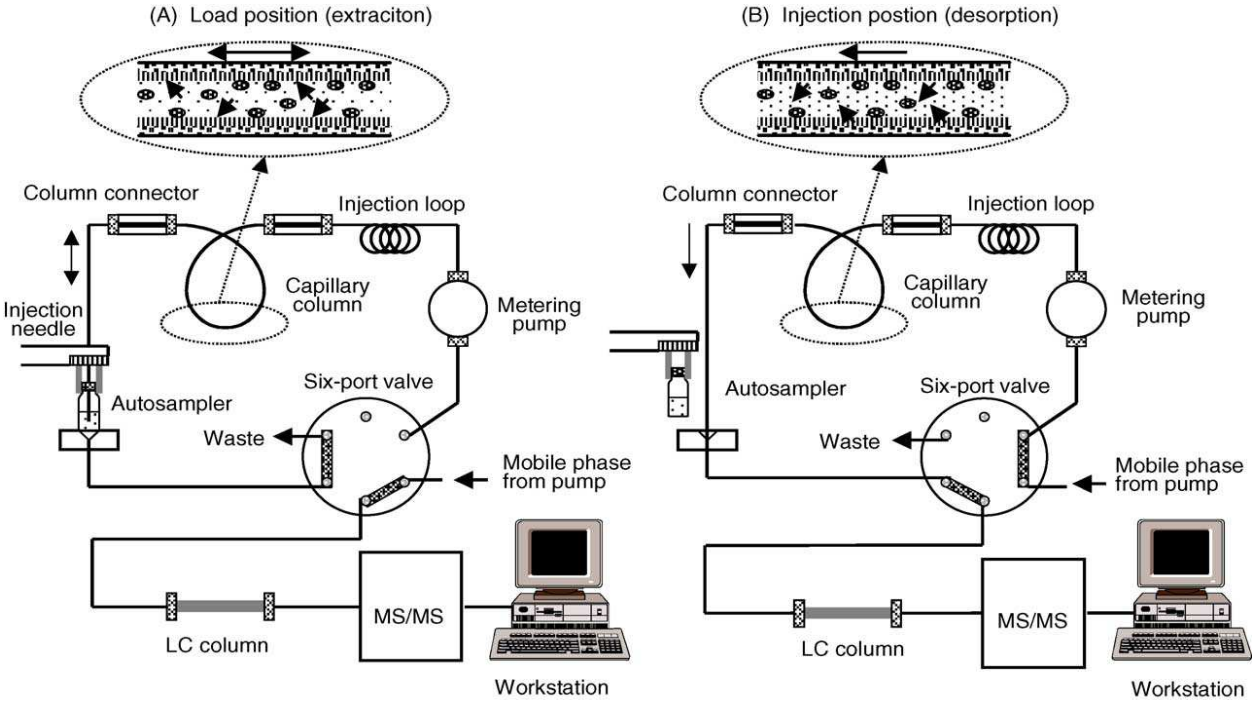

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674 Figure 7.

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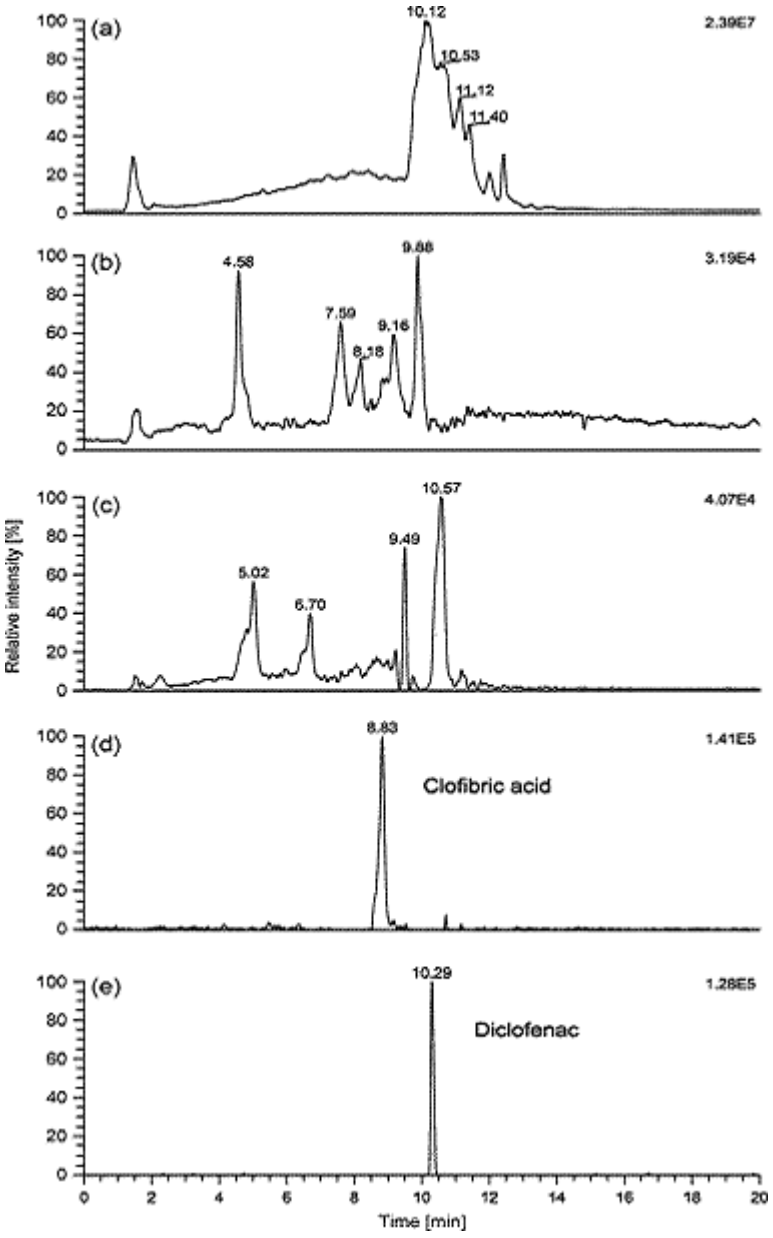
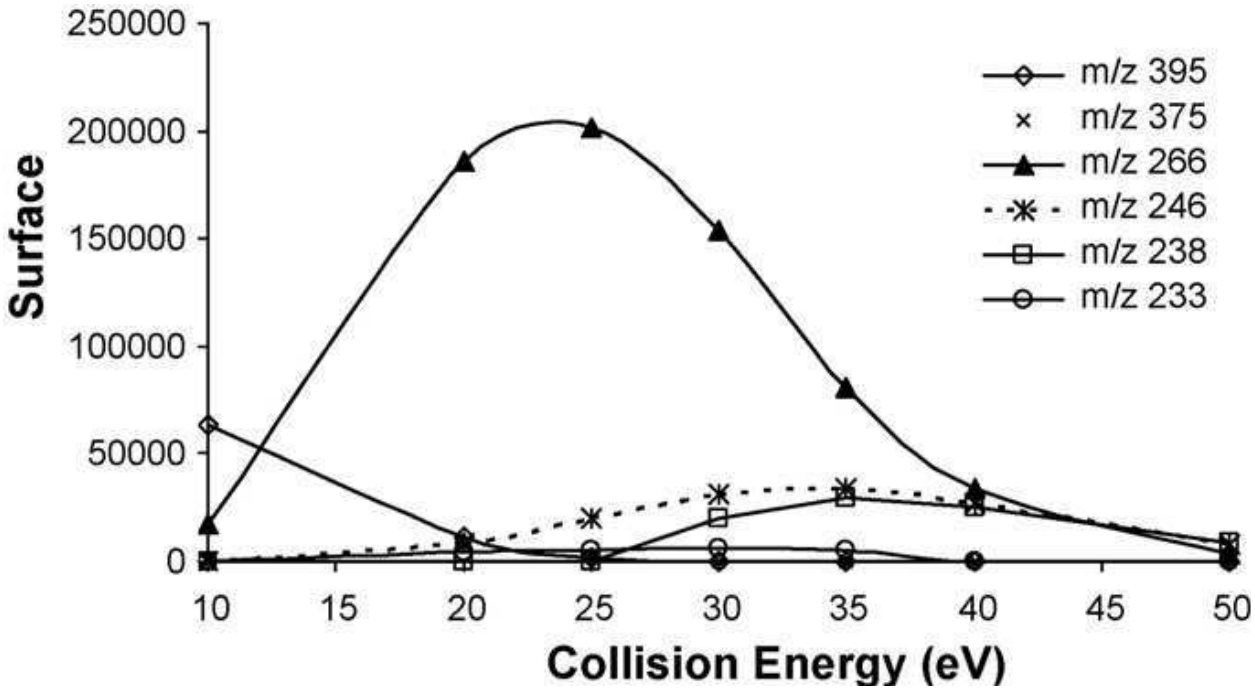


676

Figure 8.


250000
200000
150000
100000
50000
0
Surface
10
15
20
25
30
35
40
45
50
Collision Energy (eV)
m/z 395
m/z 375
m/z 266
m/z 246
m/z 238
m/z 233

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